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Biochemical Identification of *Aeromonas* Genospecies Isolated from Humans

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One hundred phenotypic characteristics were determined for 138 clinical and environmental *Aeromonas* strains. Cluster analysis revealed three major phenons equivalent to the *A. hydrophila*, *A. caviae*, and *A. sobria* groups, each of which contained more than one genospecies and more than one named species. An excellent correlation was found between phenotypic identification and classification based on DNA relatedness. DNA hybridization groups within each of the phenotypic groups were also separable by using a few biochemical characteristics. Key tests were production of acid from or growth on D-sorbitol (which separated DNA hybridization group 3 from groups 1 and 2 within the *A. hydrophila* phenogroup), growth on citrate (which essentially separated DNA hybridization group 4 from groups 5A and 5B within the *A. caviae* phenogroup), and growth on DL-lactate (which separated DNA hybridization group 1 from groups 2 and 3 within the *A. hydrophila* phenogroup as well as group 5A from groups 4 and 5B within the *A. caviae* phenogroup). All except one strain in the *A. sobria* phenogroup belonged to DNA hybridization group 8. DNA hybridization groups were not equally distributed among clinical and environmental isolates, suggesting that strains of certain DNA hybridization groups might be less virulent than others.

There is growing awareness that *Aeromonas* species are possible etiologic agents in intestinal as well as extraintestinal diseases. Most clinical laboratories, however, cannot accurately identify *Aeromonas* species. *A. salmonicida* is associated with furunculosis in salmonids (21) and is not able to grow at 37°C, and therefore, it is of no importance in clinical microbiology. The term *Aeromonas hydrophila* or *A. hydrophila* group is frequently used conveniently to include all motile, mesophilic aeromonads, which, in fact, comprise several named species (1, 12, 13, 18, 22) as well as unnamed DNA hybridization groups (16, 20). There are obvious reasons for this simplification. (i) Most commercial identification systems do not even allow identification to the level of the three phenotypically separable species *A. hydrophila* sensu stricto, *A. caviae*, and *A. sobria* as described in *Bergey's Manual of Systematic Bacteriology* (18); (ii) so far, no phenotypic tests are available that would correlate with the various DNA hybridization groups within each of the three species listed above (20); (iii) there is some question concerning the nomenclature of *A. caviae* and *A. punctata* (22); and (iv) it has been recognized only recently that the presence of potential virulence factors is correlated with certain biochemical characteristics which, in turn, are linked to the three species mentioned above (8, 15).

Since volume 1 of *Bergey's Manual of Systematic Bacteriology* was published in 1984 (22), four new species that exhibit special phenotypic characteristics have been described. These are *A. media* (1); *A. veronii* (13); *A. eucrenophila* (22), which was formerly named *A. punctata* subsp. *punctata* (21); and "*A. schubertii*" (12). Still, these species do not fully reflect the genetic heterogeneity of the genus as determined by DNA-DNA hybridization studies (12, 13, 19). Because of the lack of phenotypic characteristics that correlate with the genetic species, some of the DNA hybridization groups have not yet been named. The currently recognized species and the various DNA hybridization groups are summarized in Table 1. Throughout this report the terms *A. hydrophila*, *A. caviae*, and *A. sobria* are considered phenotypic entities as described by Popoff (18).

Some confusion occurred when ornithine decarboxylase-positive and arginine dihydrolase-negative strains were named *A. veronii*, corresponding to DNA hybridization group 10 (13). In fact, what was considered to be a separate DNA hybridization group turned out to be identical to DNA hybridization group 8 (16). Furthermore, since most, if not all, *A. sobria* strains isolated from clinical specimens also belong to DNA hybridization group 8, and because the type strain of *A. sobria* belongs to DNA hybridization group 7, clinical *A. sobria* isolates are, in fact, *A. veronii*. The striking phenotypic differences within this group may warrant splitting of the genetic species into two biotypes or subspecies.

The occurrence of genospecies in clinical (mainly fecal) specimens has been determined in only two studies, one from the United States (14) and the other one from The Netherlands (16). A genospecies or DNA hybridization group is composed of strains whose DNAs are at least 70% interrelated with 5% or less divergence within the related sequences (25). The aim of the present investigation was to determine the relative frequencies of the various DNA hybridization groups in clinical specimens in Switzerland, as well as to determine whether additional biochemical tests might be helpful for identification of these groups without the need for DNA-DNA hybridization studies, thus allowing species identification in diagnostic laboratories as well.

MATERIALS AND METHODS

***Aeromonas* strains.** One hundred two of the *Aeromonas* strains used in this study were isolated at the Department of Medical Microbiology, University of Zurich, from fecal specimens of presumably diarrheic patients. Isolation pro-

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TABLE 1. Currently recognized species and known DNA hybridization groups in the genus *Aeromonas*

Species[a]	DNA hybridization group[b]	Definition strain[c]			Comments
		Popoff	ATCC	CDC	
A. hydrophila	1	543	7966^T	9079-79	Cannot be separated phenotypically; DNA group 1 is *A. hydrophila* sensu stricto; DNA group 3 contains the type strain of *A. salmonicida*
	2	218		9533-76	
	3	316		0434-84	
A. caviae	4	545	15468^T	9083-79	Cannot be separated phenotypically; no gas from glucose; DNA group 4 is *A. caviae* sensu stricto; DNA group 5B contains the type strain of *A. media*
	5A	239		0862-83	
	5B	233		0435-84	
	6	546	23309	0859-83	*A. eucrenophila* Gas$^+$
A. sobria	7	208		9538-76	CIP 7433^T; cannot be separated phenotypically; DNA group 7 is *A. sobria* sensu stricto; DNA group 8 is *A. veronii*
	8	224		0437-84	
	9	224		0787-80	
	10/8		35624^T	1169-83	ODC$^+$ ADH$^-$[d]; *A. veronii* (see text)
	11		35941	1306-83	Phenotypically similar to *A. veronii*
	12		43700	2446-81	Mannitol$^-$, "*A. schubertii*"[e]
A. salmonicida subsp.:					
salmonicida	3		33658^T	9701-84	Nonmotile; most do not grow at 37°C
achromogenes	3		33659^T	9702-84	
masoucida	3		27013^T	9086-79	

[a] According to *Bergey's Manual of Systematic Bacteriology* (18).
[b] As defined at the Centers for Disease Control (Hickman-Brenner et al., 2nd Int. Workshop on *Aeromonas* and *Plesiomonas*, 1988).
[c] Popoff, Popoff et al. (18); ATCC, American Type Culture Collection (Rockville, Md.); CDC, Centers for Disease Control (Atlanta, Ga.); T, type strain.
[d] ODC, Ornithine decarboxylase; ADH, arginine dihydrolase.
[e] Species not yet validated.

cedures included direct inoculation of cefsulodin-Irgasan-novobiocin agar (Yersinia Selective Agar; Difco Laboratories, Detroit, Mich.), as well as enrichment in alkaline peptone water (29°C for 24 h) and phosphate-buffered saline (4°C for 14 days), followed by subculture on cefsulodin-Irgasan-novobiocin agar. Plates were incubated aerobically at 25°C for 48 h. Isolates were identified by their positive oxidase reaction after they were subcultured on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) containing 5% human blood and by a limited number of conventional biochemical tests incubated at 29°C (23). Seven strains were isolated from other human specimens, and three strains were isolated from environmental specimens. The sources of two strains were not known.

Nine reference strains representing all DNA hybridization groups that were known at the time when this study was initiated were supplied by F. W. Hickman-Brenner (Centers for Disease Control, Atlanta, Ga.). H. K. Geiss (University of Heidelberg, Heidelberg, Federal Republic of Germany) supplied 3 fecal and 10 environmental isolates as well as two strains of unknown origin. Currently recognized species and known DNA hybridization groups are listed in Table 1.

Biochemical analysis. Basic phenotypic characterization was done on 326 strains by using 11 conventional tests (23), and 69 characters were assayed in commercial kits (API 20E and API 50E; API Systems, La Balme-Les Grottes, France), as directed by the manufacturer. All tests were read after incubation at 29°C for 48 h except for those in the API 20E system, which were read after 18 to 24 h. Since some tests occurred in more than one of these systems, the actual number of different characteristics that were analyzed was only 63 (Table 2). For redundant tests, priority was given to the API 50 system followed by the API 20E system. Results for lysine decarboxylase were not included at this stage since this test was not reliable either with Falkow medium (Difco) or by the API 20E system method (2; unpublished data).

For 138 strains that differed in at least one of the basic phenotypic tests, an additional 37 characteristics were tested. These included 32 assimilation tests assayed with commercial test strips (API ATB 32GN) that were incubated at 29°C for 48 h and read automatically (3); production of hemolysin and of a CAMP-like factor, both of which were assayed aerobically and anaerobically on Trypticase soy agar (BBL) containing 5% sheep blood as described previously (10); and the presence of lysine decarboxylase activity by using the medium of Fay and Barry as described previously (2).

Cluster analysis. Pairwise unweighted similarity coefficients, S_{ij}, based on all 100 phenotypic tests were calculated with the following equation to examine the phenetic relatedness of the 138 strains: $S_{ij} = C_{ij}/(C_{ij} + D_{ij})$, where S_{ij} is the similarity coefficient, C_{ij} is the number of concordant tests between strains i and j, and D_{ij} is the number of discordant tests between strains i and j. These similarity values were clustered by using the unweighted pair group method with arithmetic averages (24).

All calculations described above were performed on a computer (Hewlett-Packard Co., Palo Alto, Calif.) by using programs written by A. Burnens (Department of Medical Microbiology, University of Zurich) in BASIC language.

DNA hybridization. The preparation and purification of DNA were essentially performed as described by Brenner et al. (4). Briefly, cells that were grown with shaking overnight at 30°C in 1.5 liters of brain heart infusion broth were harvested by centrifugation. After all the cells were completely suspended in 50 ml of lysing solution (0.05 M Tris hydrochloride [pH 8.1], 0.05 M EDTA, 0.1 M NaCl, 50 μg of pronase per ml), sodium dodecyl sulfate was added to a final concentration of 1%. The cells were allowed to lyse at 37°C and were then extracted with phenol. Sodium perchlorate (final concentration, 1.0 M) was added to dissociate the DNA and residual proteins; and after two chloroform extractions the nucleic acids were precipitated with ethanol, spooled, and dissolved in water (repeated three times). The

TABLE 2. Characteristics of the three phenons corresponding to *A. hydrophila*, *A. caviae*, and *A. sobria*

Test no.	System and test[a]	% of strains with positive characteristics (no. of strains)			
		Aeromonas spp. (138)	*A. hydrophila* (28)	*A. caviae* (78)	*A. sobria* (25)
	Conventional tests				
1	DNase	99	96	100	96
2	Indole production	93	100	90	92
3	Motility	95	96	94	96
4	Gas from D-glucose	38	82	5	80
5	Malonate	0	0	0	0
	API 20E				
6	ONPG	97	100	97	92
7	Arginine dihydrolase	90	93	85	100
8	Ornithine decarboxylase	0	0	0	0
9	Citrate	23	18	10	52
10	Hydrogen sulfide	0	0	0	0
11	Urea	0	0	0	0
12	Tryptophan deaminase	1	0	0	4
13	Voges-Proskauer	39	82	8	88
14	Gelatin	86	100	80	92
	API 50E				
15	Glycerol	98	100	96	100
16	Erythritol	0	0	0	0
17	D-Arabinose	0	0	0	0
18	L-Arabinose	82	93	96	28
19	Ribose	94	100	90	100
20	D-Xylose	0	0	0	0
21	L-Xylose	0	0	0	0
22	Adonitol	1	0	0	0
23	β-Methyl-xyloside	0	0	0	0
24	Galactose	100	100	100	100
25	D-Glucose	100	100	100	100
26	D-Fructose	100	100	100	100
27	D-Mannose	73	100	53	100
28	L-Sorbose	1	0	0	0
29	Rhamnose	6	21	1	0
30	Dulcitol	1	0	0	0
31	Inositol	0	0	0	0
32	Mannitol	99	100	100	100
33	Sorbitol	7	29	0	0
34	α-Methyl-D-mannoside	1	0	0	0
35	α-Methyl-D-glucoside	24	71	6	24
36	*N*-Acetylglucosamine	99	100	99	100
37	Amygdalin	6	4	5	0
38	Arbutin	75	96	92	0
39	Esculin	80	93	96	16
40	Salicin	72	79	91	4
41	Cellobiose	63	21	80	52
42	Maltose	100	100	100	100
43	Lactose	54	32	73	16
44	Melibiose	6	4	4	12
45	Saccharose (sucrose)	96	100	99	92
46	Trehalose	99	100	99	100
47	Inulin	0	0	0	0
48	Melezitose	1	0	0	0
49	D-Raffinose	7	0	9	8
50	Amidon (starch)	100	100	100	100
51	Glycogen	99	100	100	92
52	Xylitol	2	0	1	0
53	β-Gentobiose	30	7	47	0
54	D-Turanose	1	0	1	0
55	D-Lyxose	1	4	0	0
56	D-Tagatose	4	0	3	0
57	D-Fucose	0	0	0	0
58	L-Fucose	1	0	0	0
59	D-Arabitol	3	7	0	0
60	L-Arabitol	1	0	0	0
61	Gluconate	99	100	100	100
62	2-Ketogluconate	1	0	0	0
63	5-Ketogluconate	0	0	0	0
	API ATB 32GN				
64	Rhamnose	7	25	1	4
65	*N*-Acetylglucosamine	98	100	99	100
66	Ribose	94	100	91	100
67	Inositol	0	0	0	0
68	Saccharose (sucrose)	95	100	99	92
69	Maltose	99	100	100	100
70	Itaconate	1	0	0	4
71	Suberate	0	0	0	0
72	Malonate	0	0	0	0
73	Acetate	92	100	90	100
74	DL-Lactate	51	54	67	4
75	L-Alanine	82	96	82	32
76	5-Ketogluconate	0	0	0	0
77	Glycogen	97	100	100	92
78	3-Hydroxybenzoate	0	0	0	0
79	L-Serine	98	100	100	96
80	Mannitol	98	100	100	100
81	D-Glucose	99	100	100	100
82	Salicin	68	79	86	0
83	D-Melibiose	3	4	1	8
84	L-Fucose	1	0	0	0
85	D-Sorbitol	6	29	0	0
86	L-Arabinose	81	93	96	28
87	Propionate	19	18	22	8
88	Caprate	97	96	99	100
89	Valerate	50	71	35	76
90	Citrate	53	32	56	68
91	Histidine	99	100	100	100
92	2-Ketogluconate	0	0	0	0
93	2-Hydroxybutyrate	1	0	1	0
94	*p*-4-Hydroxybenzoate	1	4	0	0
95	Proline	100	100	100	100
	Special tests				
96	CAMP aerobic	22	93	3	4
97	CAMP anaerobic	23	96	0	16
98	Hemolysin aerobic	30	75	5	64
99	Hemolysin anaerobic	24	79	3	32
100	Lysine decarboxylase F&B	42	93	4	100

[a] Abbreviations: ONPG, *o*-nitrophenyl-β-D-galactopyranoside; F&B, Fay and Barry medium.

suspension was made to 0.1 M with respect to NaCl before each precipitation. Then, the DNA solution was made to 0.05 M with respect to Tris hydrochloride (pH 8.1), 0.1 M with respect to NaCl, and 0.5 M with respect to EDTA; and pancreatic RNase was added to a final concentration of 50 μg/ml. After incubation at 60°C for 1 h, pronase (final concentration, 50 μg/ml) and sodium dodecyl sulfate (final concentration, 1% [wt/vol]) were added, and the solution was incubated at 37°C for 60 min and then extracted once with phenol and twice with chloroform. Purified DNA was precipitated three times with ethoxyethanol and dissolved in water. A typical yield was about 5 mg of high-molecular-

weight DNA. The DNA was sheared by sonication at 4°C to a double-stranded molecular weight of about 3×10^5 (4). The concentration and purity of the DNA were assayed spectrophotometrically. DNA was labeled in vitro by using a nick-translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) and [α-^{32}P]dCTP (Dupont, NEN Research Products, Boston, Mass.) as described by the manufacturer. Labeled nucleic acids were separated from unreacted nucleotides by chromatography on a Bio-Gel P60 column (Bio-Rad Laboratories, Richmond, Calif.).

The conditions that were used to determine DNA relatedness by the hydroxyapatite method were essentially those described previously (4), except that only 75 μg of unlabeled DNA was used per assay. Relative binding ratios were determined at 75°C, 60°C, or both. The divergence within related sequences was calculated on the assumption that 1% unpaired bases within a heterologous DNA duplex decreased the thermal stability by 1°C compared with that of a homologous DNA duplex.

Generally, strains were included in a given genospecies if the DNA relatedness was above 70% at 60°C, above 60% at 75°C, or both and if divergence was less than 5%. In addition, strains that were identical by multilocus enzyme electrophoresis with 11 different enzymes (1a; M. Altwegg, A. G. Steigerwalt, J. M. Janda, and D. J. Brenner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C-253, p. 435) were also considered to belong to identical DNA hybridization groups.

RESULTS

Biochemical characterization. The unweighted pair group method with arithmetic average (24) cluster analysis of 138 *Aeromonas* strains based on 100 tests revealed three phenons and five single members or groups with no more than two members at the 88% similarity level (data not shown). The three principal phenons were equivalent to the species *A. hydrophila*, *A. caviae*, and *A. sobria* as recognized in *Bergey's Manual of Systematic Bacteriology* (18).

Of the 100 characteristics tested, six were invariably positive and 18 were invariably negative (Table 2). Of the remaining 76, only 19 exhibited major differences among the three main phenons. These were production of gas and acetoin (Voges-Proskauer) from D-glucose; aerobic and anaerobic production of hemolysin and a CAMP-like factor; decarboxylation of lysine; hydrolysis of esculin; fermentation of L-arabinose, D-mannose, α-methyl-D-glucoside, arbutin, salicin, and β-gentobiose; and growth on DL-lactate, L-alanine, salicin, D-sorbitol, and L-arabinose.

Of the 138 strains analyzed phenotypically, 78 (56.5%) clustered in the phenon corresponding to the *A. caviae* group, 28 (20.3%) clustered in the phenon corresponding to the *A. hydrophila* group, and 25 (18.1%) clustered in the phenon corresponding to the *A. sobria* group. This distribution of phenospecies mainly reflected their occurrence in fecal specimens since 104 of the 138 strains analyzed were isolated from such specimens (see Table 4).

DNA relatedness. One hundred twenty-six *Aeromonas* strains were tested for their DNA relatedness to reference strains representing the DNA hybridization groups described in Table 1. Of 72 phenotypic *A. caviae* isolates, 40 belonged to DNA hybridization group 4, 25 belonged to DNA hybridization group 5, and none belonged to DNA hybridization group 6 (Table 3). One strain each was assigned to DNA hybridization groups 1 and 2, two strains were assigned to DNA hybridization group 8, and three strains did not fall into any of the DNA hybridization groups. All but two phenotypic *A. hydrophila* strains were assigned to DNA hybridization groups 1 to 3, and all phenotypic *A. sobria* strains fell into DNA hybridization groups 7 to 9. None of our strains was a member of DNA hybridization group 11 or 12 ("*A. schubertii*," mannitol negative). The only strain that did not ferment mannitol was the type strain of *A. eucrenophila* (DNA hybridization group 6).

TABLE 3. Correlation between biochemical identification and DNA hybridization group

DNA hybridization group (no.)	No. identified as:			
	A. caviae (72)	*A. hydrophila* (26)	*A. sobria* (21)	*Aeromonas* spp. (7)
1 (12)	1	11		
2 (6)	1	5		
3 (8)		8		
4 (42)	40			2
5A (17)	17			
5B (6)	6			
5A/B[a] (2)	2			
6 (1)				1
7 (1)			1	
8 (23)	2		19	2
9 (2)			1	1
None (6)	3	2		1

[a] Cannot unequivocally be assigned to one of the two subgroups of DNA hybridization group 5.

Phenotypic separation of DNA hybridization groups. In addition to the phenotypic tests useful for identification of the three species *A. hydrophila*, *A. caviae*, and *A. sobria* (see above), only a very few tests provided a satisfactory means to separate the various DNA hybridization groups (Table 4). These included production of acid from or growth on D-sorbitol (which separated DNA hybridization group 3 from groups 1 and 2 within *A. hydrophila*), growth on citrate (which essentially separated DNA hybridization group 4 from groups 5A and 5B within *A. caviae*), and the most

TABLE 4. Biochemical tests useful for the identification of the different DNA hybridization groups occurring in clinical specimens

Test	% of strains in the following DNA hybridization groups (no. of strains)[a]:						
	1 (12)	2 (6)	3 (8)	4 (42)	5A (17)	5B (6)	8 (23)
Gas from D-glucose	75	83	75	2	6	−	83
Voges-Proskauer	67	83	75	7	12	−	78
Acid from:							
D-Sorbitol	−	17	88	5	−	−	−
Arbutin	83	+	+	93	88	+	13
Salicin	67	+	63	95	82	83	17
Cellobiose	17	67	50	71	+	83	61
Lactose	33	17	63	57	94	+	30
Growth on:							
DL-Lactate	+	−	−	95	−	+	−
Salicin	67	83	75	88	82	83	4
D-Sorbitol	−	17	88	−	−	−	−
Citrate	25	−	63	88	18	−	52
CAMP, aerobic	83	83	+	−	12	−	4
CAMP, anaerobic	83	83	+	−	−	−	17
Hemolysin, aerobic	83	+	25	−	12	−	65
Hemolysin, anaerobic	83	50	75	−	12	−	30
Lysine decarboxylase	92	+	88	−	−	−	+

[a] +, 100% positive; −, 0% positive.

TABLE 5. Source of *Aeromonas* strains in relation to DNA hybridization group[a]

Source (no.)	% of strains in the following DNA hybridization groups (no. of strains):										
	1 (11)	2 (5)	3 (7)	4 (40)	5A (16)	5B (6)	5A/B (2)	8 (20)	9 (2)	None (5)	ND[b] (13)
Feces (104)	8	3	3	34	13	6	2	17	1	4	13
Extraintestinal (6)		1		4					1		
Environmental (13)	1	1	4	1	3			2		1	
Unknown (4)	2			1				1			

[a] Reference strains for each DNA hybridization group are not included.
[b] ND, Not done.

useful single biochemical test, growth on DL-lactate (which separated DNA hybridization group 1 from groups 2 and 3 within *A. hydrophila* as well as group 5A from groups 4 and 5B within *A. caviae*). Table 4 lists all the biochemical tests that were positive for at least 80% of the strains in one DNA hybridization group and positive for less than 20% of the strains in another group.

Source of strains. Most strains analyzed in this study were isolated from human fecal specimens (Table 5). Among these, DNA hybridization groups 4, 5A, and 5B (equivalent to the *A. caviae* group) accounted for two-thirds (66 of 104) of the strains. The *A. hydrophila* group (DNA hybridization groups 1 to 3) and the *A. sobria* group (DNA hybridization groups 7 to 9) were about equally frequent, with 14 and 18 strains, respectively. Within each of the phenotypic species, the various DNA hybridization groups occurred at different frequencies. More than half of the fecal *A. hydrophila* strains (8 of 14) belonged to DNA hybridization group 1, and almost two-thirds of the fecal *A. caviae* strains belonged to DNA hybridization group 4. All except one intestinal *A. sobria* strain were members of DNA hybridization group 8. These data are in striking contrast to the frequencies of the various DNA hybridization groups for 13 environmental isolates (Table 5). For such strains, two-thirds of the *A. hydrophila* (four of six) and most of the *A. caviae* (three of four) isolates belonged to DNA hybridization groups 3 and 5A, respectively. All but one *A. sobria* isolate, regardless of source, were in DNA hybridization group 8.

DISCUSSION

Phenotypic identification. On the basis of DNA hybridization data and phenotypes, Popoff (18) has classified motile, mesophilic *Aeromonas* strains into the three species *A. hydrophila*, *A. caviae*, and *A. sobria*. These species can be identified by routine biochemical tests such as hydrolysis of esculin, production of gas and acetoin from glucose, decarboxylation of lysine, and fermentation of arabinose and salicin (5, 6, 15, 16). These results were essentially confirmed (Table 2), although the percentages for positive reactions obtained in this study were slightly different from previously published values. These variations were not unexpected since different assay systems as well as a different set of strains were used in this study. Our results, however, are not consistent with the findings of Figura and Guglielmetti (10), who described the differentiation of mesophilic *Aeromonas* strains based on differences in the production of a CAMP-like factor when grown aerobically and anaerobically. We also found that most *A. hydrophila* strains produced such a factor aerobically as well as anaerobically and that most *A. caviae* strains did not produce it at all, but we were able to detect enhancement of beta-hemolysis in only a few *A. sobria* strains when they were incubated either anaerobically or aerobically.

More than half of the mainly fecal isolates analyzed in this study were in the *A. caviae* group. This was in good agreement with the relative frequencies of occurrence of the three mesophilic species in The Netherlands (16), the Federal Republic of Germany (11), and the United States (14, 17). However, the *A. sobria* group was the species found most frequently in Australia (7).

Correlation of phenotype and DNA hybridization group. Kuijper et al. (16) have stated that phenotypic identification using the biochemical scheme of Popoff (18) for *A. hydrophila*, *A. caviae*, and *A. sobria* and the published data on *A. veronii* and "*A. schubertii*" are often inadequate in that a strain biochemically identified as one species may belong to a different genospecies. In this study, strains were classified by cluster analysis on the basis of 100 characteristics rather than by using the criteria of Popoff (18), and this resulted in an excellent correlation between biochemical identification and DNA hybridization groups (Table 3). Of 119 strains that clustered in one of the three major phenons, 5 could not be assigned to one of the known DNA hybridization groups and only 4 strains were placed in the wrong phenotypic species. These four strains were all *A. caviae* (corresponding to DNA hybridization groups 4, 5A, 5B, and 6) that were shown genetically to be *A. hydrophila* (corresponding to DNA hybridization groups 1 to 3) or *A. sobria* (corresponding to DNA hybridization groups 7 to 9). All 26 *A. hydrophila* strains as well as all 21 *A. sobria* strains were correctly identified phenotypically.

Phenotypic separation of DNA hybridization groups. While a fairly large number of biochemical tests show significant differences among the three main phenons equivalent to the species recognized in *Bergey's Manual of Systematic Bacteriology* (18), only a few tests provide a basis to separate the seven DNA hybridization groups containing the majority of clinical isolates. In contrast to recent taxonomic studies involving large numbers of phenotypic tests (5, 6, 9) that did not include DNA-DNA hybridization data, our results provide at least a partial basis for separating these seven groups (Table 4).

Within *A. hydrophila* the following tests are essential. (i) Growth on DL-lactate separates all strains of DNA hybridization group 1 from those in the other two groups; and (ii) production of acid from D-sorbitol and lactose, growth on D-sorbitol and citrate, and the hemolysis of sheep erythrocytes when incubated aerobically allow separation of DNA hybridization groups 2 and 3, respectively.

Within *A. caviae*, growth on DL-lactate was a key test in that it almost completely separated hybridization groups 4 and 5B from group 5A. Growth on citrate separated groups 4 and 5B. However, additional tests that are probably not very suitable for routine diagnostic laboratories (e.g., multilocus enzyme electrophoresis) may be needed to achieve

more reliable species identification (Altwegg et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1989).

Since all except one strain of *A. sobria* in this as well as a Dutch (16) study belonged to DNA hybridization group 8, species identification in the clinical laboratory does not cause any problems. Although *A. veronii* (ornithine decarboxylase-positive strains within DNA hybridization group 8) has been described as occurring in human fecal specimens and probably as causing diarrhea (13), they seem to be rare. None of our strains was of the ornithine decarboxylase-positive biotype. Although correct identification of most clinical *Aeromonas* isolates now seems feasible for diagnostic laboratories as well, further biochemical tests are needed to reliably identify all strains that may occur in various kinds of specimens.

Taxonomic considerations. Assuming that it is appropriate to give scientific names to each of the DNA relatedness groups (species), several problems in choosing these names are encountered. DNA hybridization group 1 would have to be named *A. hydrophila* because it contains the type strain for this species. DNA hybridization group 3 would become *A. salmonicida* because the type strain of this species has been shown to share enough DNA relatedness to strain 316 of Popoff (18) to be included in the same DNA hybridization group and, therefore, belongs to the same species (J. J. Farmer III, F. W. Hickman-Brenner, G. R. Fanning, M. J. Arduiro, and D. J. Brenner, 1st Int. Workshop on *Aeromonas/Plesiomonas*, p. P1, 1986). The striking phenotypic differences and the importance of the psychrophilic, nonmotile strains in the fish industry may warrant further separation into several subspecies, e.g., *A. salmonicida* subsp. (new name) for the mesophilic, motile strains and *A. salmonicida* subsp. *salmonicida*, *A. salmonicida* subsp. *masoucida*, and *A. salmonicida* subsp. *achromogenes* for the phenotypically separable three groups of psychrophilic, nonmotile strains. No name has yet been proposed for DNA hybridization group 2.

The proper naming of species within *A. caviae* is uncertain (22) because of the confusion introduced by the fact that two species (*A. caviae* and *A. punctata*) share a common type strain (ATCC 15468 = NCMB 74). DNA hybridization group 4 would have to be named either *A. caviae* or *A. punctata*, and group 5 would become *A. media* because the type strain of this species is in DNA hybridization group 5B (F. W. Hickman-Brenner, G. R. Fanning, M. J. Arduino, D. J. Brenner, and J. J. Farmer III, 2nd Int. Workshop on *Aeromonas* and *Plesiomonas*, p. 51, 1988). Since groups 5A and 5B are more closely related by DNA-DNA hybridization than other groups, they would have to be considered two subspecies of *A. media*, eventually as *A. media* subsp. *media* (DNA hybridization group 5B) and as *A. media* subsp. (*new name*) (DNA hybridization group 5A). Striking phenotypic differences exist between strains isolated from river water and described as *A. media* by Allen et al. (1) and the clinical strains of DNA hybridization group 5B. This fact calls for two different biogroups within *A. media* subsp. *media*. Strains of biogroup I are nonmotile and are unable to utilize sucrose as a sole carbon source (1), and strains of biogroup II are motile and are able to grow on sucrose as a sole carbon source.

The problems concerning the proper naming of *A. eucrenophila* (DNA hybridization group 6) have been discussed by Schubert and Hegazi (22). Phenotypic separation from other strains within *A. caviae* is straightforward, however, and is based on the production of gas from D-glucose.

The confusion introduced into *A. sobria* by the description of *A. veronii* has been mentioned earlier (13). The only correct solution is to name strains of DNA hybridization group 7 *A. sobria* and to treat the biochemically different strains of DNA hybridization group 8/10 as two biotypes or subspecies of *A. veronii*.

Source of strains. For fecal specimens, the relative frequencies of DNA hybridization groups found in this study compare well with those found by Holmberg et al. (14) in the United States and by Kuijper et al. (16) in The Netherlands. In all three studies DNA hybridization groups 1, 4, and 8 were the most frequent ones within the phenospecies *A. hydrophila*, *A. caviae*, and *A. sobria*, respectively. However, in our collection we found that more strains belonged to rare genospecies (DNA hybridization groups 2, 3, 5, and 9). This was probably because our strains were selected from a larger collection by excluding biochemically identical strains (based on 63 characteristics). None of the three studies revealed strains of DNA hybridization groups 6, 7, 11, or 12.

It is not known why the various DNA hybridization groups do not occur with comparable frequencies in clinical and environmental specimens (Table 3). It is doubtful, however, that the way of selecting the strains from a larger collection has any bearing on the distribution of hybridization groups. It is certainly possible that those strains that are often found in environmental samples (DNA hybridization groups 3 and 5A) are less pathogenic and, therefore, not as abundant in patients as are other groups that are believed to play a role in the pathogenesis of diarrhea. More data are needed to answer this question.

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